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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/448,356	11/23/1999	DAVID CARL BURDICK	20257/110665	4950

7590 07/14/2004

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EXAMINER	
QAZI, SABIHA NAIM	
ART UNIT	**PAPER NUMBER**
1616	

DATE MAILED: 07/14/2004

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/448,356	BURDICK ET AL.	
	Examiner	Art Unit	
	Sabiha Qazi	1616	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *28 March 2004*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-4,8,24 and 25* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-4,8,24 and 25* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1)☒ Notice of References Cited (PTO-892)
2)☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3)☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4)☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5)☐ Notice of Informal Patent Application (PTO-152)
6)☐ Other: _____.

Non-Final Action

Acknowledgment is made of the Appeal Brief filed on March 28, 2004. Finality of the action is withdrawn. Claims 1-4, 8, 24, and 25 are pending. No claim is allowed. Rejection is maintained for the same reasons as set forth in the previous Office Action.

RESPONSE TO ARGUMENTS

POINT I

THE EXAMINER RELIED ON A "SELECTION OF PRIOR ART TEACHINGS" STANDARD THAT FALLS SHORT AND IMPERMISSIBLY SHIFTS THE BURDEN TO APPELLANTS

COUNTERPOINT I

THE SELECTION OF PRIOR ART TEACHINGS CITED BY THE EXAMINER DOES NOT FALL SHORT OF THE STANDARD AND DOES NOT SHIFT THE BURDEN TO APPELLANTS

The Examiner is allowed to say, "Instant claim[s] is[are] a selection of prior art teachings..." as long as the instant claims show no criticality and/or unexpected results. Examiner agrees; it is true that most inventions are combinations of old elements, but these inventions always have something new—criticality and/or unexpected results. The instant invention does ***not*** have criticality and/or unexpected results. One would ***expect*** the same results from the prior art teachings.

After making the "combinations of old teachings" argument, Applicants attack each reference individually. Examiner will reinforce what was said in the previous Office Action: Claim 8 is rejected under 35 U.S.C. 103(a) as obvious over ***the combined teachings of*** Mitchell (US Patent 4,588,717), Kamarei et al. (US Patent 4,879,312), and Miettinen et al. (WO 92/19640). Claims 1-6, 24, and 25 are rejected under 35 U.S.C. 103 as being unpatentable ***over combined teachings*** of Miettinen et al. (WO 92/19640) and Mitchell (US 4,588,717). For example, on page 20 of the Appeal Brief, Applicants ask, "Why would one have selected DHA or EPA based on the generic disclosure of ***any*** C_{2-22} fatty acid in Miettinen?" This argument is moot because one would have selected DHA or EPA based on the generic disclosure of any C_{2-22} fatty acid in Miettinen in view of the Kamarei reference.

Examiner notes the Applicant has cited numerous case law, including *Ex parte West, Ex parte Sterner, Ex parte Bertelloti*, etc., all in support of the "combinations of old teachings" argument. To cite more case law, "One cannot show nonobviousness by **attacking references individually** where the rejections are based on combinations of references." In re Keller, 642 F.2d 413, 208 SPQ 871 (CCPA 1981); In re Merck & Co., Inc., 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986). See MPEP 2145.

On page 20, the Applicants ask, "Why would one have even considered DHA – a fatty acid containing 22 carbon atoms and within the scope of Miettinen – when Mitchell teaches that such compounds ***are not as useful*** (emphasis added) as C_{18-20} fatty acids?"

Why are the Applicants so concerned with the use? Claim 8 states, "A composition comprising…" The Applicants are claiming the ***composition***, not the ***use***.

POINT II

THE EXAMINER IMPROPERLY CONDUCTED THE REJECTION 1 ANALYSIS AT THE TIME SHE WROTE THE REJECTION

COUNTERPOINT II

THE EXAMINER PROPERLY CONDUCTED THE REJECTION 1 ANALYSIS AT THE TIME SHE WROTE THE REJECTION

On page 22 of the Appeal Brief, the Applicants quote the previous Office Action, "'[I]nstant claim ***is*** a selection of prior art teachings...'" They go on to say, "The Examiner's analysis unmistakably uses verb forms of the present and present perfect tenses – 'is,' 'would be' and 'are known.'"

Examiner would like to put emphasis on another word in that same sentence: "[I]nstant claim is a selection of ***prior*** art teachings..." Merriam Webster's Online Collegiate Dictionary (available at www.m-w.com) defines the word "prior" as "earlier in time or order."

Examiner will reinforce what was said in the previous Office Action: "Instant compositions would have been obvious **at the time of invention**." [Paper No. 17 at 8] The Examiner ***did*** "go back in time" because the quotation says that the compositions would have been obvious "**at the time of invention**."

Examiner believes this argument is not meaningful because it is based on such a small technicality. This argument is not relevant to the subject matter of the discussion. Examiner could make the same case even in this Response to Arguments. For example, three paragraphs above this one, the Examiner writes, "They go on to say..." The Examiner used "say," which is the present form of the word "said," but it does not mean that the Applicant was saying it in the present.

POINT III-VI

Similarly examiner will respond to Points III-VI at the time of writing the Examiner's answer, because arguments are same as described in our previous office actions.

Claim Rejections - 35 USC § 103

The factual inquiries set forth in *Graham* v. *John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

1. Claim(s) 1-4, 8, 24 and 25 are rejected under 35 U.S.C. 103 as being unpatentable over Higgins, III (US Patent 6,147,236) and Higashidate et al. (J. of Chromatography, 515 (1990), 295-303). These references teach sterol esters and methyl esters of eicosapentaenoic ac id (EPA) and docosahexaenoic acid (DHA), which embrace instantly, claimed invention. See the entire documents especially lines 9-67, col. 2; cols. 3 and 4; lines 1-20, col. 5 in US '236; see abstract and first Para on page 295, Table 1 and last two paragraphs on page 302 in Higashidate reference.

Instant claims differ from the reference in claiming nutritional supplement of specific sterol esters prepared by unsaturated fatty acid esters selected from EPA, DHA and Stearidonic acid (SA) whereas prior art US '236 teaches sterol esters with unsaturated fatty acids, examples

given is same as one of the instantly claimed sterol ester i.e. sterol with DHA, sitosterol docosahexaenoate and sitostanol docosahexaenoate, see lines 13 and 14 in col. 5. Higashidate teaches DHA and EPA from fish oils and prevent diseases such as arteriosclerosis and myocardial infarction by lowering the concentration of lipids and cholesterol in blood. It discloses that fish oil is a rich source of such fatty acids. Stearidonic acid (SA) is also found in fish oil.

It would have obvious to one skill in the art to prepare additional beneficial nutritional supplement using sterols with a pendent ester functionality which when hydrolyzed provides another cholesterol-lowering agent. Since Higgins teaches such sterol esters and Higashidate teaches that fish oil contains omega-3 fatty acids (a class of PUFA) which includes docosahexaenoic acid (DHA) and eicosahexaenoic acid (EPA), one would find ample motivation to prepare sterol esters with unsaturated fatty acids from active compounds present in fish oil (known to be used as nutritional supplement to loer the cholesterol and triglyceride levels) or using unsaturated fatty acids from any other source for use as nutritional supplement.

2. Claim(s) 1-4, 8, 24 and 25are rejected under 35 U.S.C. 103 as being unpatentable over combined teachings of Mitchell (US 4,588,717) and Gregory J. Mishkel et al. (Bailliere's Clinical Haematology, Vol. 3, No. 3, July 1990, pp 625-649) and Kamarei et al. (US 4879,312). See the entire documents.

Mitchell (US Patent 4,588,717) teaches vitamin supplements containing phytosterol esters such as fatty acid esters of sterol, stigmasterol and taxasterol, in various combinations, a composition of the phytosterols, such as sitosterol, stigmasterorl, taraxasterol etc. reacted with

polyunsaturated fatty acids such as linoleic acid, (18-carbons, two double bonds), linolenic acid (18-carbons, 3-double bonds), arachidonic acid (20-carbons, two double bonds). Fatty acid may have about 18-20 in addition to two carbon atoms of terminal carboxyl and methyl groups (lines 2-15, col. 6) and at least two double bonds such as arachidonic acid, linoleic acid and linolenic acids are used to make phytosterol esters, (see lines 21-58, col. 3; lines 43-65, col. 5; equation 1 and lines 1-11 in col. 8). Furthermore, it teaches that the reaction between any given phytosterol and any given fatty acid is essentially the same, and is characterized in equation 1 using sitosterol and linoleic acid as an exemplary fatty acid.

Mishkel et al. teaches that fish oil containing omega-3 fatty acids lower the serum and cholesterol levels, and their beneficial effect on preventing and treating cardiovascular disease. See 1st Para on page 626, third paragraph on page 629, second Para on page 628. Specific use of DHA and EPA as dietary supplement are disclosed on section "Angina" on page 634.

Kamarei et al. teach that a diet rich in omega-3-fatty acids has beneficial effects in humans, including a reduction in plasma cholesterol and triglyceride levels, improved fat tolerance, prolonged bleeding time reduce platelet counts and decreased platelet adhesiveness. The omega-3-fatty acids are obtained mainly from dietary seafood. It teach n-3 Poly unsaturated fatty acids (PUFA) participation and reasons why these materials may be involved in alleviating ischemic heart diseases. Furthermore, it also teaches that one of n-3 PUFA i.e. eicosapentaenoic acid (EPA) and DHA reduces triglyceride and very low-density lipoprotein (VLDL) serum levels and reduces whole blood viscosity. (See lines 39-59, col. 2; lines 13-54, col. 3; Table 1 and 2 in col. 4).

Instant claims differ from the reference in claiming nutritional supplement of phytosterol ester with specific fatty acids i.e. docosahexaenoic acid, stearidonic acid and eicosahexaenoic acid where US '717 teaches phytosterol ester with fatty acids especially containing poly unsaturated fatty acid approximately 2-22 carbon atoms. See examples 51-75 in col. 6, equation 2 in cols 15, 16, 17 and 18. Mishkel et al. teaches that polyunsaturated fatty acids from fish oil is used to preventing and treating cardiovascular disease. Furthermore, it teaches two major biologically active fish oil compounds, EPA and DHA.

Note, that Kamarie that n-3 PUFA i.e. eicosapentaenoic acid (EPA) and DHA reduces triglyceride and very low-density lipoprotein (VLDL) serum levels and reduces whole blood viscosity. (See lines 39-59, col. 2; lines 13-54, col. 3 and Table 1 and 2 in col. 4).

It would have been obvious to one skilled in the art to prepare additional beneficial nutritional supplement using sterols with a pendent ester functionality which when hydrolyzed provides another cholesterol-lowering agent. Since Mishkel teaches that fish oil contains omega-3 fatty acids (a class of PUFA) which includes docosahexaenoic acid (DHA) and eicosahexaenoic acid (EPA), see especially last para on page 625 of Mishkel reference). There has been ample motivation provided by the prior art to prepare the instant invention.

Conclusion

Since US' 236 teaches food grade sitosterol docosahexaenoate and sitostanol docosahexaenoate and other references cited above teach DHA , EPA and fish oil containing n-3 PUFA i.e. eicosapentaenoic acid (EPA) and DHA reduces triglyceride and very low-density lipoprotein (VLDL) serum levels and reduces whole blood viscosity, instant invention is considered obvious for the reasons cited above.

The compounds and compositions as claimed in present invention are considered obvious for the reasons as cited above.

Examiner notes the limitation of temperature (-20C to 20C) in claim 1. Normally, change in temperature, concentration, or both, is not a patentable modification; however, such changes may impart patentability to a process if the ranges claimed produce a new and unexpected result which is different in kind and not merely in degree from results of prior art; such ranges are termed "critical" ranges, and applicant has burden of proving such criticality; even though applicant's modification results in great improvement and utility over prior art, it may still not be patentable if the modification was within the capabilities of one skilled in the art; more particularly, where the general conditions of the claim are disclosed in the prior art, it is not inventive to discover optimum or workable ranges by routine experimentation. In re Aller et al. 105 USPQ 233.

It is well established that merely selecting proportions and ranges is not patentable absent a showing of criticality. In re Becket, 33 U.S.P.Q. 33 (C.C.P.A. 1937). In re Russell, 439 F.2d 1228, 169 U.S.P.Q. 426 (C.C.P.A. 1971).

It is a general rule that merely discovering and claiming a new benefit of an *old* process cannot render the process again patentable. Nor can patentability be found in differences in ranges recited in the claims. When the difference between the claimed invention and the prior art is some range or other variable within the claims, the applicant must show that the particular range is *critical*, generally by showing that the claimed range achieves unexpected results relative to the prior art range. In re Woodruff, 16 USPQ2d 1934.

In the light of the forgoing discussion, the Examiner's ultimate legal conclusion is that the subject matter defined by the instant claims would have been obvious within the meaning of 35 U.S.C. 103(a).

Communication

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Sabiha Qazi whose telephone number is (571) 272-0622. The examiner can normally be reached on any business day.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Kunz can be reached on (571)-272-0887. Fax phone number for the organization where this application or proceeding is assigned is 703-872-9306.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

7/10/04

SABIHA QAZI, PH.D
PRIMARY EXAMINER

Notice of References Cited	Application/Control No. 09/448,356	Applicant(s)/Patent Under Reexamination BURDICK ET AL.	
	Examiner Sabiha Qazi	Art Unit 1616	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
	A	US-5,892,068	04-1999	Higgins, III, John D.	552/554
	B	US-			
	C	US-			
	D	US-			
	E	US-			
	F	US-			
	G	US-			
	H	US-			
	I	US-			
	J	US-			
	K	US-			
	L	US-			
	M	US-			

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	Classification
	N					
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	Minskel et al. (Baillere's Clinical Haematology, Vol. 3, July 1990, pp 625-649)
	V	
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

8

Cardiovascular effects of ω-3 polyunsaturated fatty acids (fish oils)

GREGORY J. MISHKEL
JOHN A. CAIRNS

The consumption of fish has long been encouraged as a nutritious dietary source and, indeed, fish oils have been used for medicinal purposes for centuries. More recently there has been great interest in the polyunsaturated fats derived from marine animals and their possible benefits in preventing and treating cardiovascular disease. This attention extends beyond scientific inquiries, to the shelves of supermarkets and the pages of the popular press. American and British pharmaceutical firms are now producing and marketing purified and concentrated forms of these polyunsaturated fatty acids from cold-water fish.

The purpose of this chapter is to review the salient studies of fish oils and their application to human cardiovascular disease. In addition, we will review the animal models of atherosclerotic disease from which much optimism has been generated.

Glossary (Figures 1 and 2)

Fatty acid: Long carbon chain compound with a single carboxyl terminal.
Saturated fatty acid: Category of fatty acids, generally found in animal fats, which have no carbon–carbon double bonds, and act to raise total cholesterol.
Unsaturated fatty acid: Fatty acids which have at least one carbon–carbon double bond. They are identified according to the position of the first carbon double bond in relationship to the carboxyl terminal. They have variable effects on serum lipids. The ω-9 monounsaturated fatty acids, such as oleic acid, generally have a neutral effect on serum lipids.
ω-6 (n-6) Fatty acid: A class of polyunsaturated fatty acids (PUFA) which have their first carbon double bond six carbon atoms from the carboxyl terminal. The two major acids are arachidonic (AA) (the principal fatty acid constituent of animal cell membranes) and linoleic acid. Generally these compounds raise serum lipids.
ω-3 (n-3) Fatty acid: A class of PUFA which have their first carbon double bond three carbon atoms from the carboxyl terminal. This class includes α-linolenic acid and the two major biologically active fish oil compounds, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).

Baillière's Clinical Haematology—
Vol. 3, No. 3, July 1990
ISBN 0-7020-1474-5

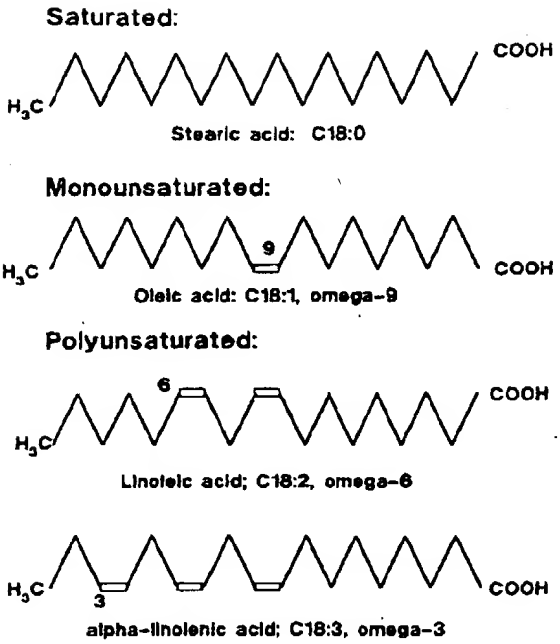

Figure 1. Diagrammatic representation of the principal classes of fatty acids. The fatty acids are named by the number of carbon atoms, next by the number of carbon–carbon double bonds, and finally the position (omega) of the first double bond as counted from the methyl terminal.

EPIDEMIOLOGY

Epidemiological surveys in the 1970s of Greenland Eskimo populations determined that cardiovascular deaths accounted for a relatively small proportion of all causes of mortality. In a 25-year mortality and morbidity study carried out on approximately 1800 Greenland Eskimos, Kronman and Green (1980) found only one case of myocardial infarction instead of the expected 40. Furthermore, the Eskimos were found to have a reduced prevalence of other chronic and inflammatory diseases, such as arthritis, psoriasis, asthma and diabetes. The age-adjusted mortality rate from myocardial infarction of these native people is estimated to be one-tenth that of their Danish neighbours to the south (Dyerberg, 1982), despite an Eskimo diet that is high in fat and cholesterol.

Eskimos are an extremely carnivorous people, with the majority of their food derived from fishing and hunting, and consisting of cold-water fish and arctic mammals. Red meat and dairy products account for only a very small proportion of their diet (Bang et al, 1976).

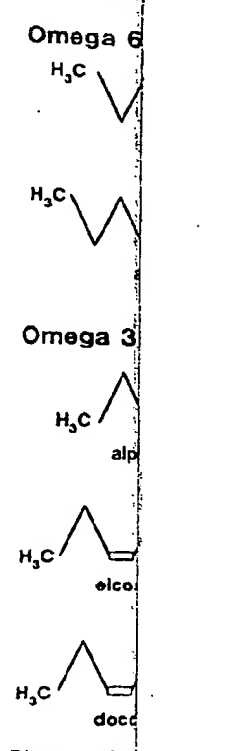

Figure 2. Diagrammatic r
fatty acids. Dietary linole
acid via desaturation.
docosahexaenoic acid, ar
from alpha-linolenic acid
sparingly, slowly, and in

This type of diet c
Furthermore, the ma
the ω-6 class charac
Greenland Eskimo
daily energy intake)
0.1 g/day (Holub, 19
this type of diet in
(Bang et al, 1971)
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alterations in eicosan
membranes (Dyerb
thromboxane A_2 (Tx
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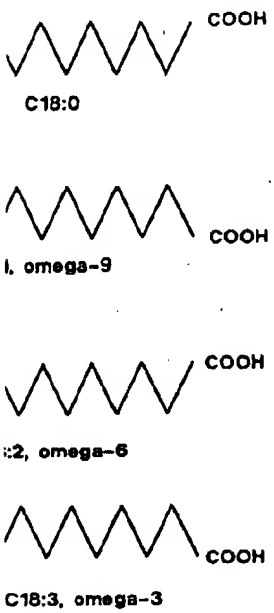
et al (1984) in a pop



ncipal classes of fatty acids. The fatty acids are the number of carbon–carbon double bonds, le bond as counted from the methyl terminal.

of Greenland Eskimo populations
is accounted for a relatively small
in a 25-year mortality and morbidity
0 Greenland Eskimos. Kronman and
myocardial infarction instead of the
nos were found to have a reduced
mmatory diseases, such as arthritis,
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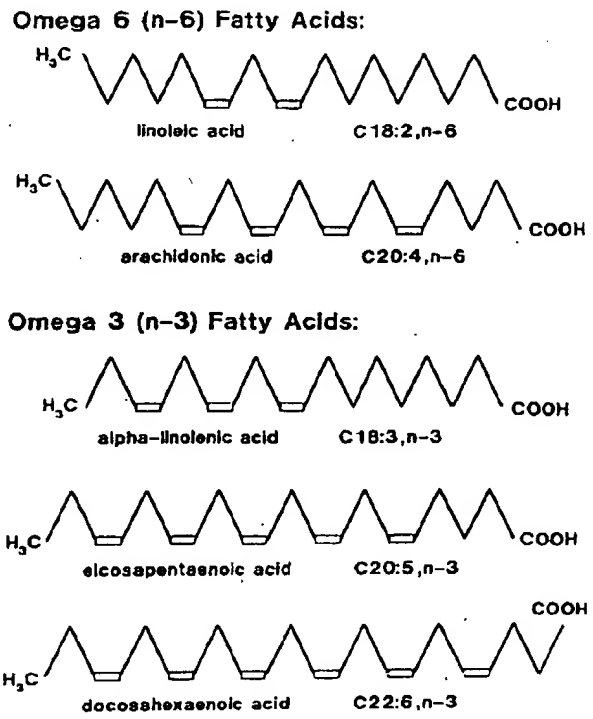


Figure 2. Diagrammatic representation of the omega-6 and omega-3 family of polyunsaturated fatty acids. Dietary linoleic acid: the parent omega-6 compound is converted to arachidonic acid via desaturation. The predominant omega-3 fatty acids, eicosapentaenoic and docosahexaenoic acid, are derived predominantly from fish oils; however, they can be derived from alpha-linolenic acid via elongation and desaturation. In humans, this is done only sparingly, slowly, and in selected tissues.

This type of diet contains a high ratio of polyunsaturated to saturated fat. Furthermore, the major polyunsaturated fat is of the ω-3 variety, rather than the ω-6 class characteristic of the North American and European diets. Greenland Eskimo adults consume approximately 4 g/day EPA (1.3% of daily energy intake), versus the North American consumption of less than 0.1 g/day (Holub, 1988). The metabolic and physiological consequences of this type of diet in Eskimos include: favourable changes in lipoproteins (Bang et al, 1971); prolongation of bleeding times, reduced platelet aggregability (Dyerberg et al, 1979) and thrombosis (Dyerberg et al, 1978); alterations in eicosanoid profiles with a lower ratio of AA to EPA in platelet membranes (Dyerberg et al, 1979); and potentially favourable shifts in thromboxane A_2 (TxA_2) and prostacyclin (Fischer and Weber, 1986). These beneficial effects in Eskimo populations have also been confirmed by Hirai et al (1984) in a population of Japanese coastal dwellers who eat 2.6 g/day

:arby dairy farming community who

dence regarding the benefit of eating
Kromhout et al, 1985). This was a
of 852 middle-aged Dutchmen who
(CAD) at the time of enrolment. It
ıse relationship between fish con-
m CAD during 20 years of follow-up.
% lower among those who consumed
ıared with those who did not eat fish,
tors. This is particularly intriguing
of 20 g/day was low in comparison to
imately 100 g/day among Japanese
ıng and Dyerberg, 1985). This study
ntake of 1 to 2 servings per week may

fish consumption and subsequent
ınfirmed by a brief Swedish report
ıation of the Western Electric study
dy, the 25-year risk of death from
dose-dependent fashion, from 20.5%
13% when more than 35 g/day of fish
ɜ epidemiological surveys have not
ı fish ingestion and the reduction of
Heart Study (Curb and Reed, 1985)
ɜn et al, 1987) are noteworthy in this
results may stem from differences in
ietary history, or cultural and genetic
though no direct link between the
eduction in cardiovascular mortality
ıvide a consistent link between the
ıhysiological effects reported in the
ets rich in fish (Kagawa et al, 1982),
: al, 1983), and arctic mammals (Bang

ue regarding the fatty acid membrane
t disease. Prisco et al (1986) reported
rd dietary regimen, with either stable
ıls. Levels of platelet membrane AA
ıower in patients with unstable angina
h no coronary lesions. Levels of AA
correlated with TxA_2 production.
ionship between the fatty acid com-
: membranes and the relative risk of
ıl study of patients with new onset of
ıl infarction. Platelet EPA concentra-
:tor for angina but not for myocardial

FISH OILS AND ATHEROSCLEROSIS

The pathogenesis of atherosclerosis, as described by Ross (1986), involves the interactions of four types of cells: monocytes, platelets, endothelial cells and smooth muscle cells, as well as non-cellular plaque constituents such as proteoglycans, fibrous tissue, calcium and collagen.

Dietary intake of ω-3 fatty acids has the potential to modify a number of these interactions and therefore to be more effective in the prevention and treatment of vascular disease. The effect of ω-3 fatty acids on platelet, endothelial cell, monocyte or red cell function in relationship to thrombogenesis is discussed in detail in an accompanying chapter.

Clinical trials of fish oils in the prevention of human coronary thrombosis and atherosclerosis have yet to be published; however, a large body of animal data now exists. Weiner et al (1986) assessed the effect of dietary ω-3 fatty acid supplementation in a hyperlipidaemic swine model of coronary atherosclerosis. These animals manifest coronary disease that is nearly equivalent to humans with respect to its pathology and clinical sequelae, as well as possessing similar platelet and coagulation systems. The swine were placed on a high cholesterol diet for 3 weeks and then underwent balloon injury to the left anterior descending coronary artery. For the next 8 months they were maintained on the high cholesterol diet and were randomized to receive 30 ml of cod liver oil (3.6 g EPA) daily or placebo. In both groups, total plasma cholesterol, low-density lipoproteins (LDL), very low-density lipoproteins (VLDL) and high-density lipoproteins (HDL) rose significantly. After 8 months, the fish oil group had an EPA : AA ratio approaching the levels typically seen in Eskimos, and serum TxA_2 levels had fallen significantly. Only minimal disease was seen in the coronary artery specimens from the fish oil treated animals, whereas all of the control animals had moderate to severe intimal hyperplasia. There was significantly less coronary atherosclerosis in the cod liver oil fed animals than in the controls, as indicated by mean lesion area, mean percentage of luminal encroachment and mean maximal luminal encroachment (Figure 3). In the control group, quantifiable microscopic calcium was significantly higher and, although only one vessel had been injured, all three coronary arteries had similar disease severity. The beneficial changes seen with the fish oil were independent of any positive effect on lipids, but correlated with easily detectable changes in levels of platelet arachidonate and EPA, and serum TxA_2.

Shimokawa and Vanhoutte (1988) studied an analogous porcine model of atherosclerosis. It differed, however, in that it provided an earlier stage of coronary atherosclerosis because cholesterol feeding continued for 10 weeks rather than 8 months. In the cod liver oil group, there was significantly less atherosclerotic disease in the balloon-injured left anterior descending artery, and milder intimal lesions in the uninjured right coronary artery (exposed to hypercholesterolaemia alone) as compared to the cholesterol fed group. This antiatherogenic benefit was independent of any favourable changes in cholesterol.

The authors, however, proposed an alternative explanation for the anti-

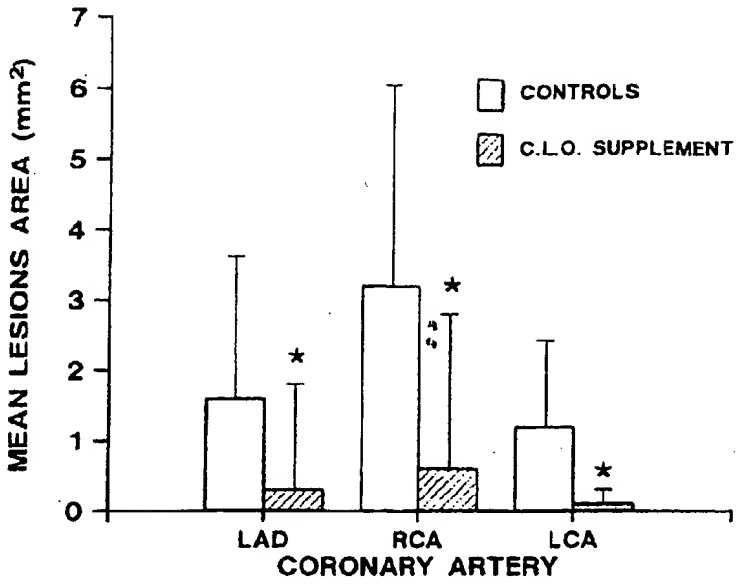
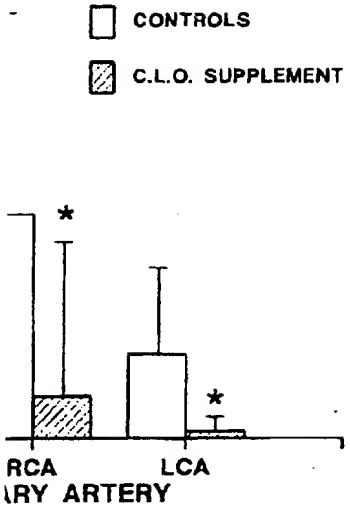


Figure 3. Comparison of the degree of atherosclerosis in the coronary arteries of hyperlipidaemic swine. Only the left anterior descending (LAD) artery underwent balloon injury, although similar degrees of atherosclerosis developed in the right coronary artery (RCA) and left circumflex artery (LCA). After 8 months of 3.6 g/day EPA, there was a dramatic and significant reduction in the mean lesion area in all arteries, as well as the mean and maximal luminal encroachment (not shown), compared with controls (★, $P = 0.05$). C.L.O., cod liver oil. From Weiner et al (1986).

atherosclerotic effects of EPA. Endothelium-derived relaxant factor (EDRF) is not only a vasodilator but also a potent inhibitor of platelet aggregation. In control animals, impaired endothelium-dependent relaxations were observed in the injured atherosclerotic left anterior descending artery and to a lesser degree in the uninjured hypercholesterolaemic right coronary artery. However, animals fed cod liver oil had normalization of endothelial-dependent responses in the uninjured hypercholesterolaemic artery, and improvement in the impaired responses in the injured atherosclerotic artery to the levels obtained with hypercholesterolaemia alone. Bioassays revealed that the improved relaxation by the dietary treatment was associated with the enhanced release of EDRF. These results, together with similar findings in normal animals (Shimokawa and Vanhoutte, 1989), suggest that dietary treatment with fish oil may protect endothelial function by augmenting the relaxation response to aggregating platelets.

Kim and colleagues (1989) confirmed these observations in a similar hypercholesterolaemic swine model but made two further other important observations. Fish oils significantly reduced the number of smooth muscle cells and monocyte-macrophages in these atherosclerotic lesions when



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compared with the untreated control pigs. Hyperlipidaemic rabbits fed fish oil supplemented diets (Thiery and Siedel, 1987) have elevated plasma levels of the products of lipid peroxidation, measured as thiobarbituric acid-reactive substances (TBARS). These substances, which are associated with diets rich in unsaturated fatty acids, can induce cellular injury. Kim also detected TBARS, however, since the atherosclerotic lesions were *smaller* and showed *less necrosis* in the treated groups: their presence apparently had no adverse effects.

These latter studies documented the beneficial effects of fish oils on the progression of atherosclerosis. Further to this, Sassen et al (1989), in a similar hyperlipidaemic-injury swine model, demonstrated that fish oils can also lead to *regression* of coronary atherosclerosis.

Davis et al (1987) documented reduction of atherosclerosis in a Rhesus monkey model which had dietary substitution of ω-3 fatty acids for coconut oil. These authors also demonstrated that the fish oil group had fewer macrophages in the atherosclerotic lesions, perhaps as a result of the putative anti-inflammatory effects of ω-3 fatty acids. Cholesterol remained quite elevated in the fish oils group, although less so than in the controls.

The results of studies in rabbit models of atherosclerosis are inconsistent with observations of disease inhibition (Zhu et al, 1988), disease acceleration (Thiery and Seidel, 1987), and no difference in disease (Rich et al, 1989). Discrepancies in this animal model may derive from several sources. Studies often differ as to the breed of rabbit used: some investigators use a breed that requires cholesterol feeding to promote atherosclerosis, while others use homozygous or heterozygous Wantanbe heritable spontaneously hyperlipidaemic rabbits. Calcium antagonists, for instance, have also been demonstrated to have divergent effects on atherogenesis, depending on the rabbit model used. Species differences might also account for the differences in the biological effects of fish oil. For example, fish oil has been reported actually to enhance monocyte adhesion and fatty streak formation (the initial step in atherosclerosis) in the rat (Rogers and Karnovsky, 1988). However, in models of atherosclerosis which most closely simulate the human situation, a beneficial effect independent of any lipid has been consistently observed.

POTENTIAL CARDIOVASCULAR USES FOR FISH OILS

From these basic animal and human studies, a variety of potential therapeutic uses in humans emerge.

Hyperlipidaemia

Dyerberg et al (1975) found that levels of total cholesterol and LDL cholesterol were lower, and that HDL cholesterol were higher among the Greenland Eskimos as compared with their age and sex-matched Danish counterparts. Recent studies have demonstrated that fish oils are effective in lowering elevated triglycerides (TG) and VLDL by 30–80% (Phillipson et

al, 1985). The reduction in VLDL may be a consequence of reduced hepatic synthesis of TG (Harris, 1984a), increased clearance by tissues (Harris, 1984b) or increased biliary secretion (Connor et al, 1981). Fish oils can modestly elevate HDL cholesterol (Holub et al, 1987) and increase the number of HDL receptors and the turnover of HDL (Roach et al, 1987).

The beneficial effects on atherogenic LDL cholesterol are less consistent. Earlier studies reported favourable effects (Nestel et al, 1984); however, more recent reports (Sullivan et al, 1986; Reis et al, 1988) have demonstrated paradoxical elevations in LDL cholesterol and apoprotein B (apo-B). Davidson and colleagues (1988) have suggested that this discrepancy is due to the high cholesterol and saturated fat content of the fish oil supplements being used. Harris et al (1988) tested this hypothesis in a placebo controlled trial, using a supplement which contained no cholesterol or saturated fat, in patients with type IV (elevated TG) or type IIb (elevated TG and cholesterol) hyperlipoproteinaemia. Administration of ω-3 fatty acids indeed lowered TG and VLDL in all of the hypertriglyceridaemic patients. However, the LDL cholesterol and apo-B levels were raised in patients with previously high or normal levels. It is probable that the earlier studies demonstrating a lowering of LDL cholesterol were a result of a combination of reduced saturated fat intake and a large increase in the intake of polyunsaturated fats.

A role for ω-3 fatty acids in the therapy of lipid disorders is therefore probably restricted to patients with hypertriglyceridaemia, where a consistent benefit is demonstrated. Caution is advised however, because of the potential offsetting effects of raising LDL cholesterol and apo-B levels.

Hypertension

The ingestion of fish oils, and even fish flesh, has been associated with a reduction in blood pressure. Early reports indicated a hypotensive effect even with ω-6 PUFA. However, because of variations in study design and the complex nature of dietary influences on human physiology, reports in the literature are often at variance.

Singer and colleagues (1986) placed patients with mild essential hypertension (< 100 mmHg diastolic) on a diet supplemented with 2.2 g/day EPA and then 3.3 g/day EPA. They observed a significant decline in systolic and diastolic pressures when compared to controls, even with the lower weekly intake. These changes occurred after only 2 weeks of intake, with values returning to baseline within 2 months of cessation. The blood pressure lowering effects can also be demonstrated in patients with secondary causes of hypertension, such as end-stage chronic renal failure requiring haemodialysis (Rylance et al, 1986), as well as in healthy volunteers (Rogers et al, 1987).

As these hypotensive changes occur without major shifts in plasma catecholamines (Singer, 1986) or the renin–aldosterone system (Mortensen et al, 1983), they have been tentatively attributed to enhanced synthesis of prostacyclins and reduced synthesis of TxA_2. Knapp and Fitzgerald (1989) examined this hypothesis by assessing the endogenous production of prosta-

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cyclin, prostaglandin E_2 (PGE_2) and thromboxane in males with essential hypertension, during a 4-week period of low (1.8 g/day EPA) and high dose (9.0 g/day EPA) fish oil supplementation, as well as a diet of ω-6 PUFA (39 g/day safflower oil) and another diet of a mixture of oils approximating the types of fat present in the American diet.

Only the high dose fish oil group had any significant reduction of systolic or diastolic blood pressure during the study period. Although the total production of prostacyclins (PGI_2 and PGI_3) increased during the first week, during the subsequent 3 weeks, while blood pressure was falling, the total amount of prostacyclin was falling in parallel to baseline levels. Urinary metabolites of thromboxane, however, did fall in a dose-related fashion in both fish oil groups. The excretion of the vasodilatory hormone, PGE_2, demonstrated a non-significant downward trend during fish oil supplementation. These authors concluded that the fall in thromboxane production and changes in prostacyclin synthesis were unlikely to have led to blood pressure lowering, although it is possible that changes in prostacyclin synthesis induced other, as yet undescribed, changes in blood pressure control.

Vascular disease

Symptomatic vascular disease is not determined solely by the degree of arteriosclerotic lumenal narrowing but also by ongoing thrombotic processes characterized by platelet–vessel wall interactions. The net functional result of these changes, in patients with atherosclerosis who have dietary supplementation with fish oils, is complex and is just becoming apparent. Knapp et al (1986) studied the effects of fish oil on the potential for modification of platelet–vessel wall interactions in patients with advanced peripheral vessel disease. These patients have elevated basal levels of TxA_2 and prostacyclin metabolites in their urine as compared with healthy controls, presumably as a result of ongoing platelet–vessel wall interactions. When they were fed 10 g/day EPA for 4 weeks, there was a significant fall in the vasoconstrictor TxA_2, associated with a minor accumulation of inactive TxA_3 metabolites. Concomitant with these changes, PGI_2 fell to control values, and PGI_3 levels rose significantly. Bleeding times were prolonged by 30% and favourable trends in parameters of in vitro platelet aggregation were observed. However, this effect on platelet aggregation is less than that observed after the administration of a single 325 mg dose of aspirin because the residual 10% capacity to form thromboxane is sufficient to sustain the aggregation process (Reilly and FitzGerald, 1987).

Hay and coworkers (1982) found, in a population of patients with ischaemic heart disease, a reduction in the plasma level of β-thromboglobulin after fish oil supplementation, together with evidence of prolonged platelet survival. Healthy individuals (Ahmed and Holub, 1984), haemodialysis patients (Rylance et al, 1986), stroke victims (Green et al, 1985) and diabetics (Haines et al, 1986) all demonstrate these potentially beneficial alterations in platelet–vessel interactions after fish oil supplementation.

Claudication

It is known that EPA reduces whole blood viscosity, as well as increasing erythrocyte deformability (Terano et al, 1983). Woodcock and associates (1984), in a randomized control trial, demonstrated a reduction in whole blood viscosity in patients with peripheral vascular disease who received 1.8 g/day EPA for several weeks. These changes were attributed to the incorporation of EPA into the erythrocyte membrane, as there was no reduction in packed cell volume or plasma viscosity. Bruckner et al (1987) demonstrated that healthy volunteers given fish oil supplements, as compared with those given olive oil, evidenced an increase in capillary blood flow velocity by a factor of 1.75. The clinical efficacy of fish oil supplementation on claudication has not yet been defined.

Angina

Clinical studies examining the effects of fish oils on coronary artery disease are rare. Saynor et al (1984) looked at the relatively long-term effects (up to 2 years) of dietary supplementation with 3.8 g/day EPA in 31 patients who had symptomatic angina. Although there were no objective data to quantitate reduction in myocardial ischaemia, and no controls, the number of anginal attacks experienced fell, and the nitroglycerin consumption declined from almost 30 tablets to only 5 tablets per week. Accompanying this fall was a favourable effect on lipid and bleeding profiles.

Mehta et al (1988) reported on the use of 3.2 g EPA/2.2 g DHA per day, in a double blind, crossover, placebo controlled study of symptomatic stable angina. Treadmill testing during the period of fish oil supplementation led to favourable, but non-significant, trends in the time to onset of ST segment depression (4.1 ± 2.8 to 6.5 ± 3.8 min) and the exercise time to angina (5.0 ± 3.5 to 5.4 ± 2.5 min) when compared with baseline. Furthermore, a highly significant ($P < 0.01$) reduction in rest and exercise double product was observed during fish oil supplementation, and non-significant trends were observed in weekly anginal frequency ($P < 0.6$) and nitroglycerin consumption ($P < 0.2$). However, the study included only eight patients.

Myocardial infarction

Although early reports of experimental myocardial infarction in the rat suggested that diets supplemented with fish oil could have an arrhythmogenic effect and lead to a larger infarct (Gudbjarnassen and Halligrimsson, 1975), more recent reports indicate benefit in pretreatment with ω-3 fatty acids.

In a canine model of myocardial infarction induced by electrical stimulation of the left circumflex artery (Culp et al, 1980), dogs fed for 36–45 days prior to the event maintained a more normal ECG pattern, and demonstrated less than 30% ectopic beats after 19 h. The control animals had 80% ectopic beats during the same time. Additionally, the fish oil fed dogs had smaller areas of infarction (3% of the left ventricle) when compared to

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controls (25%). The authors were unable to explain adequately the apparent protective effect of the fish oils. The work of Benedict et al (1989) suggests a possible explanation. In his model of spontaneous thrombosis of a circumflex artery, dogs fed 400 mg kg^{-1} day^{-1} total ω-3 PUFA demonstrated significantly less vasospasm in the circumflex artery ($P<0.01$), as well as less platelet aggregation as measured by peak serotonin levels. Perhaps the reported reduction in plasminogen activator inhibitor that occurs with ω-3 PUFA intake also acts to decrease thrombosis and maintain vessel patency (Mehta et al, 1989).

In an experimental model of circumflex artery occlusion followed by *reperfusion* (Oskarsson et al, 1988), fish oil fed dogs had significantly smaller infarcts (13 ± 3% of area at risk) versus controls (29 ± 7%). The difference in infarct size could not be attributed to different areas at risk or changes in collateral blood flow. Malis et al (1988), having demonstrated reduced vasoconstriction and enhanced relaxation in isolated rat aorta preparations exposed to anoxia, postulated that these favourable changes in vascular contractility may be the mechanism for improved postischaemic reperfusion in this animal model.

Another potential explanation for these findings was provided by Hock and colleagues (1987) who demonstrated that dietary supplementation with fish oils leads to profound changes in the fatty acyl composition of the phospholipids in sarcolemmal membranes, with a reduction of the ω-6 : ω-3 ratio. They also demonstrated a reduction of infarct size after coronary artery ligation, as measured by a reduction in the loss of creatine kinase. They postulated that these membrane changes could affect cellular transport processes or enzyme activities, and could lead to alterations in cardiac function under physiological and pathophysiological conditions such as myocardial ischaemia. Although functional parameters have not been examined in the hearts of animals fed fish oils, past studies support the principle that dietary modification of myocardial lipids is associated with changes in myocardial function. For instance, animals fed sunflower oil demonstrate an increased coronary flow rate (DeDeckre and ten Hoor, 1980), as well as diminished contractile responses to various inotropes (Dryden et al, 1982).

Another potential benefit of long-term fish oil supplementation is its effect on arrhythmias during myocardial infarction, and particularly during reperfusion. McLennan and associates (1988) examined a whole animal model of arrhythmia and sudden cardiac death, produced by ligating the left anterior descending coronary artery of anaesthetized rats, and subsequently allowing reperfusion by releasing the ligature. Animals were pretreated for 12 months with one of four diets: standard rat chow, or the same diet supplemented with ω-6 PUFA (sunflower seed oil), or a saturated fatty acid diet (sheep fat), or an ω-3 PUFA diet (tuna oil). The incidence of ventricular fibrillation was virtually zero during the period of occlusion with the sunflower oil and the tuna oil diets, whereas the incidence was significantly increased with the sheep fat diet when compared with the standard reference diet. A reduction in the incidence and duration of ventricular tachycardia was also observed. Furthermore, during the period of reperfusion, the fish oil diet totally abolished ventricular fibrillation, although the other diets

either had no effect or were deleterious (sheep fat). It appears that there are differences between the types of unsaturated fatty acids in dietary lipids in relation to their effects on arrhythmia development. This is possibly explained by the incorporation of ω-3 fatty acids into myocardial phospholipids leading to decreased production of TxA_2, a compound which has been implicated in reperfusion arrhythmias (Coker and Parratt, 1985).

Benediktsdottir and Guobjarnason (1985) also demonstrated that rats consuming cod liver oil were less prone to ventricular fibrillation after isoprenaline than those that ate maize oils. However, in the only human study published to date, Hardarson et al (1989), using a crossover design, found that 20 ml/day cod liver oil begun 1 week after uncomplicated myocardial infarctions had no effect in reducing the number of ventricular extrasystoles as compared to placebo. This was a relatively small study of only 18 men and, unlike the animal models administration, took place under relatively stable ischaemic conditions.

To date there have been no human studies to determine the effect of fish oils on infarct size reduction, primarily because of the period of pretreatment required. However, a British randomized controlled trial with a factorial design (Burr et al, 1989) has recently examined the possibility that dietary changes can reduce the risk of myocardial infarction recurrence and death. Two thousand and thirty-three men who had recovered from a myocardial infarction were allocated to receive or not to receive three sets of dietary advice: a reduction in fat intake and an increase in the ratio of polyunsaturated to saturated fats; an increase in fatty fish intake; and an increase in dietary cereal fibres. There were eight possible combinations of these dietary factors, including one group who received no dietary advice of any kind.

Those patients who received advice regarding increased fish consumption had a 29% reduction in all-cause mortality, the difference being attributable to a reduction in deaths related to ischaemic heart disease. This significant benefit appeared early, after 3 months, and persisted for up to 2 years, and was independent of other confounding factors. However, there was no reduction in the rates of non-fatal reinfarction, and in neither of the other two dietary groups was any benefit observed.

Objective evidence of compliance with dietary advice was obtained from serum cholesterol and plasma fatty acid measurements. The amount of fish consumed was small, contributing approximately 2.5 g EPA weekly, and was not associated with favourable changes in cholesterol status.

Cerebral vascular accidents

No controlled studies have reported on the effects of fish oils on strokes in humans; however, the protective effect of ω-3 fatty acids on acute cerebral ischaemia has been investigated in an animal model (Black et al, 1979). For 18–24 days prior to ligation of the middle cerebral artery, cats were fed either cat chow alone or supplemented with ω-3 PUFA. This prophylactic administration resulted in a lesser neurological deficit and volume of brain infarction. Analysis of the lipid content of those cats fed the ω-3 PUFA diet

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indicated no changes in brain tissue; the authors thus concluded that this apparent prophylactic benefit accrued due to beneficial changes in eicosanoid profiles, with elimination of secondary cerebral vascular spasm.

Coronary artery bypass grafts

Coronary artery bypass grafting (CABG) has now been performed for the last 20 years, with vein graft occlusion accounting for the greatest postoperative morbidity. The highest risk of closure occurs during the first month postoperatively, as a result of graft thrombosis. Over the first year, an accelerated form of narrowing occurs because of intimal hyperplasia; thereafter, vein grafts undergo an atherogenic process indistinguishable from that in arterial lesions. The overall result is that about 10 years after CABG, approximately 50% of grafts are occluded.

Therapy with aspirin alone (Goldman et al, 1988) or in combination with dipyridamole (Chesboro et al, 1982) begun prior to CABG in humans has been demonstrated to reduce significantly the incidence of acute occlusion, due to thrombosis, and those occurring during the first year postoperatively, due to intimal hyperplasia. Animal studies suggest that this intimal hyperplasia is likely to be platelet mediated, and have confirmed that a combination of aspirin and dipyridamole can reduce its incidence.

Cod liver oil has also been shown to reduce intimal hyperplasia in autologous vein grafts used for arterial bypass. In a dog model receiving a 2% cholesterol diet for 1 week prior to the interposition of an autologous jugular vein between bilaterally divided femoral arteries, Landymore and colleagues (1985) observed a 350% reduction in the degree of intimal hyperplasia seen in a cod liver oil treated group of animals when compared with controls. Using the same model, Landymore et al (1986) also looked at a direct comparison of fish oil versus a combination of aspirin and dipyridamole (Figure 4). The intimal thickness increased approximately twentyfold after 6 weeks of the high lipid diet in control animals. Aspirin–dipyridamole significantly reduced this intimal hyperplasia by approximately 50% at sacrifice, whereas the fish oil further reduced it by 75% ($P<0.004$). Casali et al (1986), interposing synthetic arterial grafts, similarly demonstrated that mackerel fed dogs had improved graft patency and reduced intimal hyperplasia at the anastomotic site when compared to dogs on a regular diet or those supplemented with aspirin–dipyridamole.

Cahill and colleagues (1988), using a similar venoarterial autograft canine model, concluded that the beneficial effects of fish oils on graft intimal hyperplasia was mediated by decreased TxA_2 production. A cholesterol diet, with or without EPA supplementation, did not affect PGI_2 production (measured by radioimmunoassay) in any native vessel or the vein grafts. EPA in this canine model does not appear to increase hepatic LDL receptors or significantly alter lipid levels.

Researchers from the Mayo Clinic postulated an alternative mechanism (Komori et al, 1989) using a porcine model fed a 2% high cholesterol diet, with or without cod liver oil supplementation. In porcine femoral veins, as in porcine coronary arteries (Shimokawa et al, 1987), cod liver oil facilitates

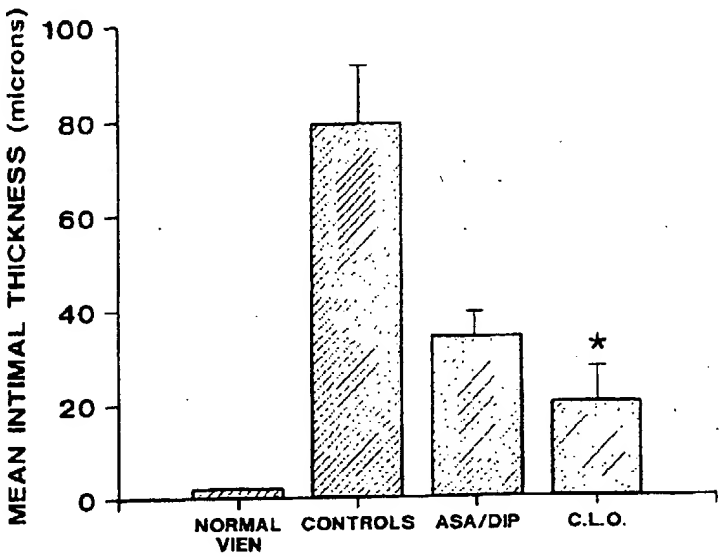
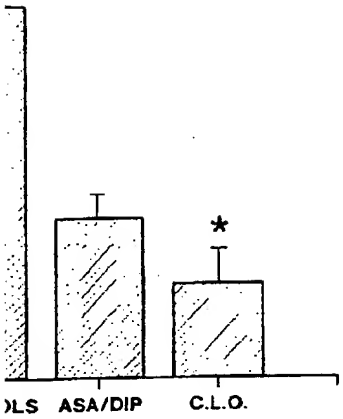


Figure 4. A comparison of control dogs fed a 2% cholesterol diet for 1 week prior to jugular vein grafting to divided femoral arteries, contrasting the supplemental effects of 30 mg kg^{-1} day^{-1} aspirin and 25 mg kg^{-1} day^{-1} dipyridamole (ASA/DIP), and cod liver oil (C.L.O.) to provide 1.8 g/day of EPA. At 6 weeks sacrifice there is significant intimal hyperplasia (83 ± 10 microns) compared to normal jugular veins (4.5 ± 0.2). Both medical regimens reduced this intimal hyperplasia ($P < 0.001$); ASA/DIP to 37 ± 3 microns, and EPA significantly more so to 24 ± 2.5 (*, $P = < 0.004$). From Landymore et al (1986).

endothelium-dependent relaxations in response to platelets, serotonin, and adenosine diphosphate. This reduction in the vasoconstriction induced by aggregating platelets is independent of any changes in vasodilator prostaglandins. The fact that cod liver oil supplementation promotes the release and synthesis of EDRF may be the reason why ω-3 PUFA decrease the intimal hyperplasia in canine venous autografts.

The combination of aspirin and fish oils on venoarterial autografts has also been evaluated. In one such controlled study (Sarris et al, 1989a), (1) fish oils, (2) aspirin, (3) the thromboxane synthetase inhibitor CGS-12970, (4) fish oil plus aspirin, and (5) fish oils plus CGS-12970, were evaluated for their effects on thromboxane generation and the degree of subsequent intimal thickening in cholesterol fed dogs. At 3 months, a similar degree of suppression of platelet aggregation was observed in all experimental groups as compared with controls, although there was a significant diminution in thromboxane generation only in the dogs fed aspirin, and a complete abolition in those dogs receiving aspirin plus fish oils. A significant reduction in intimal hyperplasia was observed only in those dogs receiving fish oils, with none further from the addition of aspirin. A weak reduction in in vivo serum (but not plasma) growth factor activity associated with fish oil



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treatment was suggested in this experiment. This altered mitogenic activity correlated with the quantitative degree of reduction of vein graft intimal hyperplasia.

Rigorous trials of ω-3 fatty acid supplementation in humans undergoing bypass surgery have not yet been reported. In one preliminary report (DeCaterina et al, 1988), 15 patients received 3 g/day EPA for 28 days prior to CABG. The anticipated changes in platelet and lipid profiles were observed. Spontaneous prostacyclin production by the saphenous veins was greater after EPA than in controls.

Although these animal studies taken together clearly demonstrate that ω-3 PUFA administration will predictably and significantly reduce the degree of intimal hyperplasia associated with venous bypass grafting, the exact mechanism of action by which EPA (and DHA) exert this beneficial effect remains unclear.

Cardiac allograft arteriosclerosis

Accelerated allograft coronary artery disease is one of the most frequent causes of late death in human cardiac transplantation. Although similar in many respects to 'natural' atherosclerosis, this process differs in that it develops rapidly and in a diffuse obliterative manner. When standard anti-rejection agents are administered, the addition of aspirin, with or without dipyridamole, does not reduce the incidence or degree of accelerated allograft coronary disease. DeCampli et al (1988) looked at the effects of cod liver oil on dogs undergoing venoarterial allografting. No significant differences were found between controls, aspirin–dipyridamole treated and cod liver oil treated animals with respect to the degree of subendothelial myointimal cell proliferation caused by chronic or recurrent acute immunological rejection.

In this study, the dogs were not on any immunosuppression, so that the extensive immune-mediated endothelial damage that occurs during the first 3 weeks of venoarterial allografting in dogs may have been overwhelmingly strong. It is possible that fish oils may become effective late after transplantation when endothelial integrity is re-established. Also, the use of antirejection agents, a more clinically analogous situation, may have helped reduce the degree of intimal thickening. Indeed, Sarris et al (1989b) demonstrated that in a rat model of cardiac allografts treated with cyclosporine, fish oil supplementation was significantly superior in reducing the degree of allograft coronary disease when compared to controls or those treated with aspirin and dipyridamole.

Percutaneous transluminal coronary angioplasty

Percutaneous transluminal coronary angioplasty (PTCA) is a highly successful, safe and cost-effective means of achieving myocardial revascularization. However, 6 months after angioplasty, 30–40% of patients have developed restenosis at the site of the dilatation. The incidence has changed little since the introduction of PTCA. The process is presumably initiated by injury to the

intima and media which occurs during balloon inflation. Within hours of the procedure there is a dense deposition of platelets, followed by monocytes, at the site of dilatation. During the healing phase, smooth muscle cells migrate from the media and proliferate, presumably in response to growth factors secreted by platelets and macrophages which have accumulated at the site of injury.

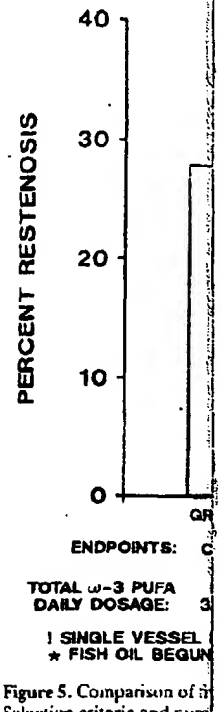
A variety of potentially beneficial pharmacological interventions have produced disappointing results. These include: various antiplatelet agents, anticoagulant regimens, thromboxane synthetase inhibitors, and prostacyclin derivatives. Because restenosis, like atherosclerosis, probably results from an interplay of various biological pathways, an agent directed at only one component of the cascade is unlikely to be successful. Theoretically, fish oils could be of benefit because of their multifactorial actions on various components of this injury process.

Lam and colleagues (1987) have demonstrated that dietary supplementation with fish oil can diminish platelet–arterial wall interaction in vivo after vessel injury. Normolipaemic pigs, receiving dietary supplementation with 4 weeks of fish oil, underwent carotid artery injury by balloon angioplasty. When compared with controls (no fish oil diet), these animals demonstrated significantly less platelet deposition and injury-related vasoconstriction at the sites of deep arterial injury. Reduced platelet deposition was demonstrated, not only in vivo but also ex vivo, when blood from these pigs was superfused at a controlled shear rate on to normal untreated porcine aortic media. However, fish oil, like other platelet inhibitors, including aspirin, did not prevent the monolayer of platelet adhesion at sites of mild injury.

Five trials of fish oils for the prevention of restenosis after PTCA (Figure 5) have been reported. The issue remains unsettled because each of these trials has different inclusion and exclusion criteria, and only two have relatively complete angiographic follow-up. Different dosage regimens, and, in particular, different durations of fish oil supplementation prior to the PTCA to allow for incorporation into cellular membranes, have been employed.

Three trials used either clinical parameters to define restenosis or had incomplete angiographic follow-up. Slack and colleagues (1987) studied 162 patients who underwent PTCA and received a total of 1.8–2.7 g/day fish oils (1.1–1.6 g/day EPA). Clinical assessment and treadmill testing were done every 2 months for 6 months after the procedure. In the 113 patients who had a single lesion dilated, clinical evidence of restenosis occurred in 16% who had taken EPA versus 33% of the controls. However, no such benefit could be demonstrated for the 49 patients with multivessel PTCA.

Milner and associates (1989) assessed clinical restenosis and angiograms, when indicated, in 183 patients over a 6-month period. One group received a total of 4.5 g/day fish oil (3.2 g EPA) and had a restenosis rate of 19% at 6 months, compared with a rate of 36% in the control group ($P < 0.008$). This favourable result is surprising in light of the fact that these patients were not treated with the fish oil prior to their PTCA, receiving the supplement within 24 h *after* the procedure. In the 45 patients whose restenosis was proved by



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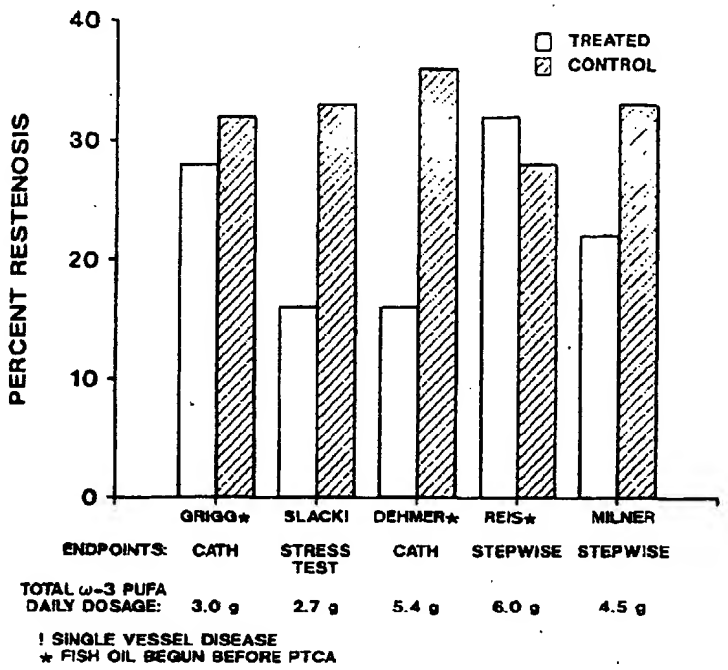

Figure 5. Comparison of five trials of fish oils to reduce the incidence of restenosis post PTCA. Selection criteria and number of patients in each trial, as well as the total daily fish oil dosage, length of pretreatment, and concomitant medications varies from trial to trial. Follow up angiography as an end-point in the detection of restenosis was used in only two trials in over 90% of patients entered. One trial used only an ischaemic response on treadmill testing as an indication of restenosis and two trials proceeded stepwise from treadmill testing to coronary angiography only if indicated. PUFA, polyunsaturated fatty acid.

angiography there was a non-significant trend favouring those subjects who took fish oils (18 versus 27%, $P<0.12$).

In a contradictory study (Reis et al, 1989) of 222 patients, there was no difference in the overall clinical restenosis rate at 6 months between the fish oil treated group as compared with controls (35 versus 24% respectively). However, symptoms and treadmill tests were used as an end-point, with only 37% of patients having their anatomy confirmed by follow-up angiography. In these patients, too, there was a trend towards a higher restenosis rate in the fish oil treated group (33%) versus controls (23%). This study is difficult to interpret because of various design problems. The first 28 patients randomized were treated in an unblinded way. Patients received a total of 6 g/day fish oil, but as two different preparations, either constituted as an ethyl ester or as a triglyceride. Furthermore, the length of pretreatment varied from 1 to 7 days (mean 5.4 ± 3.1 days).

Only two studies have had complete follow-up angiography performed.

Grigg et al (1989) detected no difference in restenosis rates in 104 patients randomized to 3.0 g/day fish oil versus placebo. There was angiographic follow-up in 93% of the patients between 3 and 4 months. However, a relatively low dose of 1.8 g/day EPA was used, and the medication was started only within the first 24 h prior to the procedure.

Dehmer et al (1988) studied 83 patients who received either a standard PTCA regimen (aspirin–dipyridamole) or, in addition, 5.4 g/day fish oil (3.2 g/day EPA) begun 1 week prior to the PTCA. One hundred per cent angiographic follow-up was obtained at 3–4 months, documenting a highly significant 77% reduction in restenosis rates, in the fish oil-treated group. During clinical follow-up over the next 12 months, after the fish oils were discontinued, no new symptomatic events suggestive of restenosis were observed.

This study was also important in documenting that ω-3 fatty acids could be used in combination with two other antiplatelet agents without causing significant prolongation of bleeding times, clinically significant thrombocytopenia, or haemorrhagic complications related to the PTCA procedure or subsequent emergency CABG. Caution must be used in generalizing from this small study as it was performed on a highly selected population of male veterans, 56% of whom smoked.

Fish oil supplementation appears promising for the prevention of post-angioplasty restenosis, but larger and more generalizable studies with more complete angiographic follow-up are required to evaluate the role of fish oils in the prevention of post-PTCA restenosis.

PHARMACODYNAMICS

Dosages

Incorporation kinetics reveal that EPA ingested as the ethyl ester appears in plasma free fatty acids and plasma phospholipids within 4 h of ingestion, but is not incorporated into platelet membranes in amounts sufficient to produce measurable changes in function until day 6 (Von Schacky and Weber, 1985). It appears that platelet membrane fatty acid composition does not immediately reflect that of the plasma milieu but rather is determined during megakaryocyte maturation.

How much fish oil must be taken daily to achieve desirable effects is unclear. Eskimos ingest 5–10 g/day EPA over a lifetime, but smaller intakes may be beneficial as prophylaxis against vascular disease. Over-cooking, deep-frying, salting or pickling of fish will reduce the amount of available EPA and DHA.

Consumption to 2–5 g/day ω-3 PUFA is likely to be sufficient to induce changes in prostaglandin and lipoxygenase products. The amount of ω-3 fatty acids required to effect changes in cellular membranes appears to be less than that required to lower lipids. For instance, initial dosages to lower serum triglycerides must be in the range of 5–10 g/day, with overall results being dose dependent. Changes occur within the first 2–6 weeks after

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starting therapy. It has been demonstrated that fish oil concentrate at high doses (10 g/day ω-3 PUFA) followed by reduced dosages (6 g/day) cannot sustain the initial lowering of plasma TG (Schectman et al, 1989). Moreover, the effect of the higher dose appears to tail off after the first month of therapy.

The dose required to reduce blood pressure consistently is uncertain. Whereas 1.8 g/day EPA was insufficient in Knapp's study (1989), such dosages have previously been reported to be effective (Mortensen et al, 1983; Houwelingen et al, 1987). From the reports in the literature one can expect falls of approximately 5–10% in both systolic and diastolic pressures, equivalent to the effects of a thiazide diuretic or β-blocker.

Of the fish oil supplements currently on the market, the most palatable are the concentrated forms in gelatin capsules. These preparations have been highly purified and refined and contain 300–500 mg of ω-3 fatty acids. Cod liver oil, which contains 20% ω-3 fatty acids, must be treated to remove excess vitamins A and D, and to reduce cholesterol and pesticide residues. Fish oil supplements are generally well tolerated, even though daily consumption of 10–20 capsules is necessary. The major side-effects are gastrointestinal and include fishy taste, belching, flatulence, bloating and occasionally diarrhoea. Newer, more concentrated compounds are being developed, with more than 500 mg EPA per capsule, allowing for fewer capsules per day and improved compliance.

POTENTIAL TOXICITY

Bleeding complications

Because of the prolongation of bleeding times and the mild thrombocytopenia that has occasionally been reported, there are concerns regarding bleeding complications, particularly the risk of haemorrhagic strokes. Eskimos reportedly have an increased incidence of cerebral 'apoplexy' (Kronman and Green, 1980), although objective evidence of intracerebral bleeding has not been documented. Furthermore, epidemiological surveys have demonstrated a reduced incidence of all strokes in populations ingesting large amounts of fish oils (Hirai et al, 1984). Clinical trials using these agents, even in combination with aspirin, have not generally reported any additional untoward effects. Although isolated reports of significant thrombocytopenia have been documented, these episodes have generally occurred with higher doses than are currently being used in clinical trials, where this has not been a reported problem.

Immune system

There is a theoretical concern regarding an increased risk of cancer because of auto-oxidation and lipid peroxidation, with the production of carcinogenic byproducts. Toxic lipid peroxides, such as malondialdehyde, have been found in cod liver oil (Piché et al, 1988). However, vitamin E, as an

anti-oxidant, is added to all capsule preparations used for public sale and in clinical trials. None of the epidemiological studies previously cited have demonstrated a basis for this concern. Greenland Eskimos and the Japanese have amongst the lowest documented rates of breast cancer. In addition, there is animal work which suggests that ω-3 fatty acids have a protective effect against experimental cancers of the pancreas (O'Connor et al, 1985), breast (Jurkowski and Cave, 1985) and colon (Reddy and Sugie, 1988).

Although not observed clinically, there exists the theoretical possibility of an immunodeficiency state produced by chronic fish oil administration. These concerns exist because of the observation in ω-3 PUFA treated patients of the reduction in levels of the mediators of inflammation and immune responses such as interleukin-1 (Endres et al, 1988), as well as transient declines in whole blood neutrophil counts, aggregation and chemotaxis (Lee et al, 1985; Mehta et al, 1988). A recent account (Ogden et al, 1988) reported an unusual case of reactive lymphoid hyperplasia that occurred with the ingestion of fish oils, and resolved promptly after discontinuation, only to recur almost immediately after rechallenge. Lymph node biopsy demonstrated reactive hyperplasia with prominent histiocytosis.

Metabolic

Besides the previously described elevations in atherogenic LDL cholesterol and apo-B, adverse effects on blood sugar homeostasis have been reported. In patients with type II diabetes mellitus, Glauber and colleagues (1988) have reported a fall in plasma insulin levels leading to an increase in blood sugar concentrations. After a diet supplemented for 1 month with 5.5 g/day ω-3 fatty acids, fasting glucose rose from 13.1 ± 1.3 to 15.3 ± 1.3 mmol/litre ($P = 0.03$). This effect was reversed when fish oils were stopped. Kasim et al (1988) confirmed this finding, and additionally demonstrated that in type II diabetic patients without overt hyperlipidaemia, ω-3 PUFA administration is associated with a potentially detrimental rise in apo-B concentrations.

The trend towards decreased PGE_2 production (Knapp and Fitzgerald, 1989) needs to be further explored. If this relationship holds true, then there is the potential for inducing or worsening renal failure in susceptible populations by suppressing renal production of this vasodilatory hormone. This would be of particular concern in the elderly with borderline renal function, and in those with renovascular hypertension or hypertension related to intrinsic renal disease. In a rat model (Scharschmidt et al, 1987), where residual renal function is dependent exclusively on prostaglandin production, renal failure has developed after the administration of fish oils.

CONCLUSION

The theoretical and experimental benefits of ω-3 fatty acid dietary supplementation, and the positive results of clinical studies, permit cautious optimism about a future beneficial role for these agents in the primary and

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REFERENCES

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secondary prevention of atherosclerosis. It is likely that the beneficial influences operate independently of any changes in lipid profiles. These agents have an additional advantage because of their lack of serious toxicity. Further, well controlled clinical trials are required to confirm the positive findings in the initial human trials of the beneficial effects of fish oils on PTCA restenosis and bypass graft occlusions, the possible interactions with other therapeutic agents, and adverse metabolic effects.

Acknowledgements

This work was supported in part by a grant from the Canadian Heart Foundation.

REFERENCES

Ahmed AA & Holub BJ (1984) Alteration and recovery of bleeding times, platelet aggregation and fatty acid composition of individual phospholipids in platelets of human subjects receiving a supplement of cod-liver oil. *Lipids* 19: 617–624.

Bang HO & Dyerberg J (1985) Fish consumption and mortality from coronary heart disease. *New England Journal of Medicine* 313: 823.

Bang HO, Dyerberg J & Nielson AB (1971) Plasma lipid and lipoprotein patterns in Greenlandic west-coast Eskimos. *Lancet* i: 1143–1145.

Bang HO, Dyerberg J & Hjørne N (1976) The composition of food consumed by Greenland Eskimos. *Acta Medica Scandinavica* 200: 69–73.

Bang HO, Dyerberg J & Sinclair HM (1980) The composition of the Eskimo food in north western Greenland. *American Journal of Clinical Nutrition* 33: 2657–2661.

Benedict D, Sheng WL & Todd GE (1989) Dietary supplementation with fish oil prevents coronary occlusion by thrombus formation. *Journal of the American College of Cardiology* 13: 195 (abstract).

Benediktsdottir VE & Guobjarnason S (1985) Dietary cod-liver oil modifies sarcolemmal lipid composition and reduces the incidence of ventricular fibrillation in rats. In Marcuse R (ed.) *Proceedings of the Thirteenth Scandinavian Symposium on Lipids* (Rekyavik), pp 26–30.

Black KL, Culp B, Madison D et al (1979) The protective effects of dietary fish oil on focal cerebral infarction. *Prostaglandins and Medicine* 3: 257–268.

Brox JH, Killie J-E, Gunnes S & Nordy A (1981) The effect of cod liver oil and corn oil on platelets and vessel wall in man. *Thrombosis and Haemostasis* 46: 604–611.

Bruckner G, Webb P, Greenwell L et al (1987) Fish oil increases peripheral capillary blood cell velocity in humans. *Atherosclerosis* 66: 237–245.

Burr ML, Gilbert JF, Holliday RM et al (1989) Effects of changes in fat, fish, and fibre intakes on death and myocardial reinfarction: diet and reinfarction trial (DART). *Lancet* ii: 757–761.

Cahill PD, Sarris GE, Cooper AD et al (1988) Inhibition of vein graft intimal thickening by eicosapentaenoic acid: reduced thromboxane production without change in lipoprotein levels or low-density lipoprotein receptor density. *Journal of Vascular Surgery* 7: 108–117.

Casali RE, Hale JA, LeNarz L et al (1986) Improved graft patency associated with altered platelet function induced by marine fatty acids in dogs. *Journal of Surgical Research* 40: 6–12.

Chesboro JH, Clements IP, Fuster V et al (1982) A platelet-inhibitor-drug trial in coronary-artery bypass operations: benefit of perioperative dipyridamole and aspirin therapy on early postoperative vein-graft patency. *New England Journal of Medicine* 307: 73–78.

Coker SJ & Parratt JR (1985) AH23848, a thromboxane antagonist, suppresses ischaemia and reperfusion-induced arrhythmias in anaesthetized greyhounds. *British Journal of Pharmacology* 86: 259–264.

Connor WE, Lin DS & Harris WS (1981) A comparison of dietary polyunsaturated ω-6 and ω-3

gland Journal of Medicine 312: 1217–1224.
telet function, thromboxane formation and
ion of the Western diet with cod liver oil.

; (1988) Dietary fish oil prevents ventricular
n and reperfusion. *American Heart Journal*

8) Effects of dietary fish oils on vascular
n 78(supplement II): 216 (abstract).
y supplementation with ω-3 polyunsaturated
irt disease. Effects on indices of platelet and
:. *American Journal of Medicine* 84: 45–51.
in plasminogen activator inhibitor-1 (PAI-1)
intake. *American Heart Journal* 116: 1201–

989) Usefulness of fish oil supplements in
percutaneous transluminal coronary angio-
94–299.
:rberg J (1983) The effect of *N*-6 and *N*-3
food lipids and blood pressure. *Thrombosis*

ppression by diets rich in fish oil of very low
al of Clinical Investigation 74: 82–89.
h consumption and mortality from coronary
6.
ell TC (1985) Effect of dietary intake of fish
ascrine-induced preneoplastic lesions in the
nstitute 75: 959–962.
1 DF (1988) Reactive lymphoid hyperplasia
Annals of Internal Medicine 109: 843–844

988) Dietary supplementation with fish oil
pplement II): 216 (abstract).
t al (1985) Reduction of plasma lipids.
oils in patients with hypertriglyceridemia.
1216.
ialdehyde excretion by subjects consuming
is. *Lipids* 23: 370–371.
sed thromboxane A_2 generation and altered
from patients with active angina pectoris.

els of omega-3 and omega-6 fatty acids on
F344 Rats. *Cancer Research* 48: 6642–6647.
romboxane formation in vivo and ex vivo:
ry drugs. *Blood* 69: 180–186.
ish oil supplements raise LDL cholesterol
patients with coronary disease. *Circulation*

andomized trial of fish oil for prevention of
li: 177–181.
elopment of atherosclerosis in genetically
ingestion. *Arteriosclerosis* 9: 189–194.
Fish oil down-regulates the low density
h density lipoprotein receptor of rat liver.

oil enhances monocyte adhesion and fatty
rat. *American Journal of Pathology* 132:

ts of a fish oil supplement on serum lipids,

blood pressure, bleeding time, haemostatic and rheological variables. *Atherosclerosis* 63: 137–143.

Ross R (1986) The pathogenesis of atherosclerosis: an update. *New England Journal of Medicine* 314: 488–500.

Rylance PB, Gordge MP, Saynor R et al (1986) Fish oil modifies lipids and reduces platelet aggregability in haemodialysis patients. *Nephron* 43: 196–202.

Sarris GE, Fann JI, Sokoloff MH et al (1989a) Mechanisms responsible for inhibition of vein-graft arteriosclerosis by fish oil. *Circulation* 80(supplement I): 109–123.

Sarris GE, Mitchell RS, Billingham ME et al (1989b) Inhibition of accelerated cardiac allograft arteriosclerosis by fish oil. *Journal of Thoracic and Cardiovascular Surgery* 97: 841–855.

Sassen LMA, Hartog JM, Lamers JMJ et al (1989) Mackerel oil and atherosclerosis in pigs. *European Heart Journal* 10: 838–846.

Saynor R, Verel D & Gillot T (1984) The long-term effect of dietary supplementation with fish lipid concentrate on serum lipids, bleeding time, platelets and angina. *Atherosclerosis* 50: 3–10.

Scharschmidt LA, Gibbons NB, McGarry L et al (1987) Effects of dietary fish oil on renal insufficiency in rats with subtotal nephrectomy. *Kidney International* 32: 700–709.

Schectman G, Kaul S, Cherayil GD et al (1989) Can the hypertriglyceridemic effect of fish oil concentrate be sustained? *Annals of Internal Medicine* 110: 346–352.

Shekelle RB, Missell LV, Paul O et al (1985) Fish consumption and mortality from coronary heart disease. *New England Journal of Medicine* 313: 821.

Shimokawa H & Vanhoutte PM (1988) Dietary cod-liver oil improves endothelium-dependent responses in hypercholesterolemic and atherosclerotic porcine coronary arteries. *Circulation* 78: 1421–1430.

Shimokawa H & Vanhoutte PM (1989) Dietary N-3 fatty acids and endothelium-dependent relaxations in porcine coronary arteries. *American Journal of Physiology* 256: H968–H973.

Shimokawa H, Lam JYT, Cheseboro JH et al (1987) Effects of dietary supplementation with cod liver oil on endothelium-dependent responses in porcine coronary arteries. *Circulation* 76: 898–905.

Simonsen T, Vartun A, Lyngmo V & Nordoy A (1987) Coronary heart disease, serum lipids, platelets, and dietary fish in two communities in northern Norway. *Acta Medica Scandinavica* 222: 237–245.

Singer P, Berger I, Lück K et al (1986) Long-term effect of mackerel diet on blood pressure, serum lipids and thromboxane formation in patients with mild essential hypertension. *Atherosclerosis* 62: 259–265.

Sluck JD, Pinkerton CA, Van Tassel J et al (1987) Can oral fish oil supplement minimize restenosis after PTCA? *Journal of the American College of Cardiology* 9: 64 (abstract).

Sullivan DR, Sanders TAB, Trayner IM & Thompson GR (1986) Paradoxical elevation of LDL apoprotein B levels in hypertriglyceridemic patients and normal subjects ingesting fish oil. *Atherosclerosis* 61: 129–134.

Terano T, Hirai A, Hamazaki T et al (1983) Effect of oral administration of highly purified eicosapentaenoic acid on platelet function, blood viscosity and red cell deformability in healthy human subjects. *Atherosclerosis* 46: 321–331.

Thiery J & Seidel D (1987) Fish oil feeding results in an enhancement of cholesterol-induced atherosclerosis in rabbits. *Atherosclerosis* 63: 53–56.

Von Schacky C & Weber PC (1985) Metabolism and effects on platelet function of the purified eicosapentaenoic and docosahexaenoic acids in humans. *Journal of Clinical Investigation* 76: 2446–2450.

Weiner BH, Ockene IS, Levine PH et al (1986) Inhibition of atherosclerosis by cod liver oil in a hyperlipidemic swine model. *New England Journal of Medicine* 315: 841–846.

Wood DA, Butler S, MacIntyre C et al (1987) Linoleic and eicosapentaenoic acids in adipose tissue and platelets and risk of coronary heart disease. *Lancet* i: 177–182.

Woodcock BE, Smith E, Lambert WH et al (1984) Beneficial effect of fish oil on blood viscosity in peripheral vascular disease. *British Medical Journal* 288: 592–594.

Zhu B-Q, Smith DL, Sievers RE et al (1988) Inhibition of atherosclerosis by fish oil in cholesterol-fed rabbits. *Journal of the American College of Cardiology* 12: 1073–1078.

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